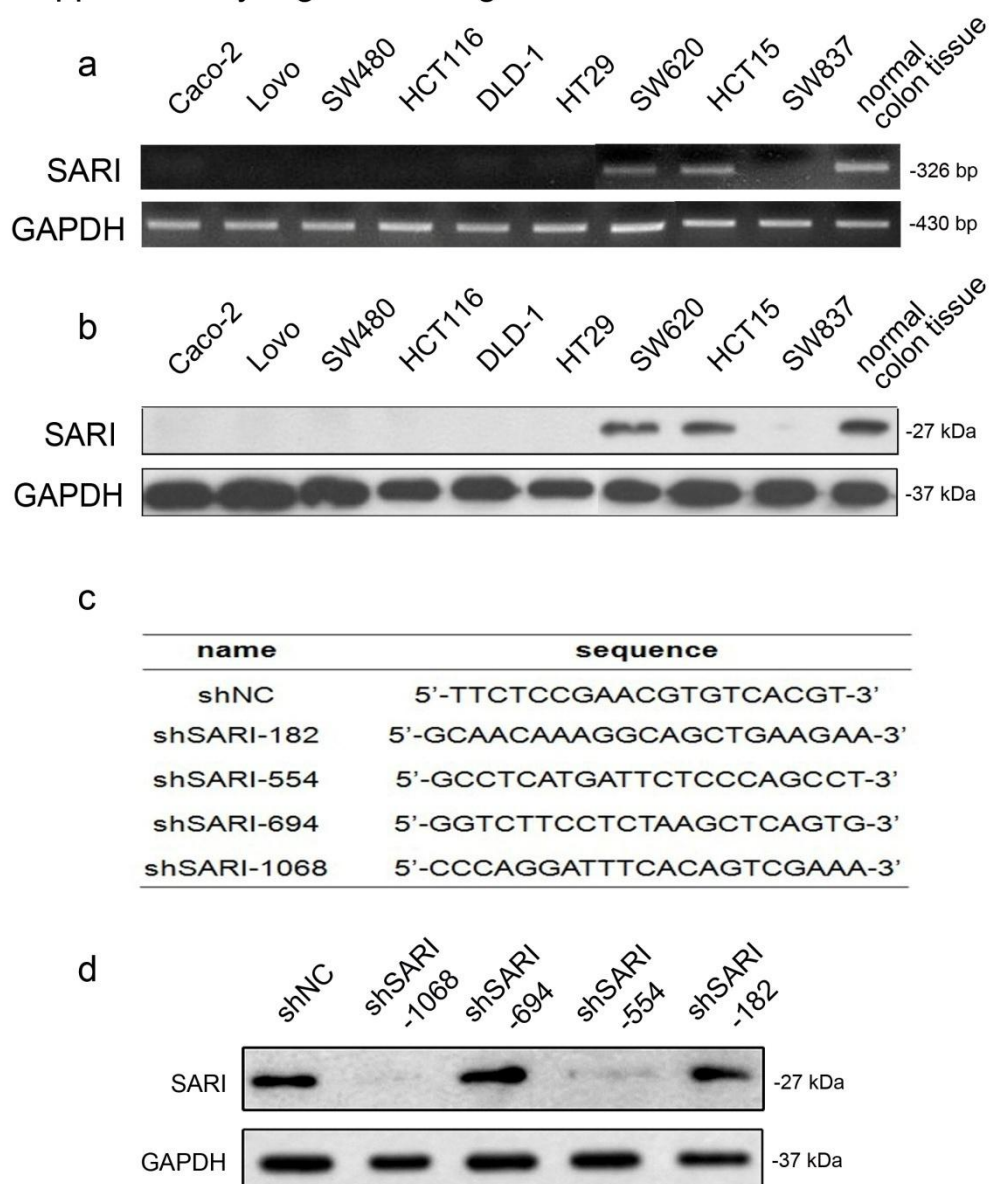


## Supplementary Figure-1 Deng

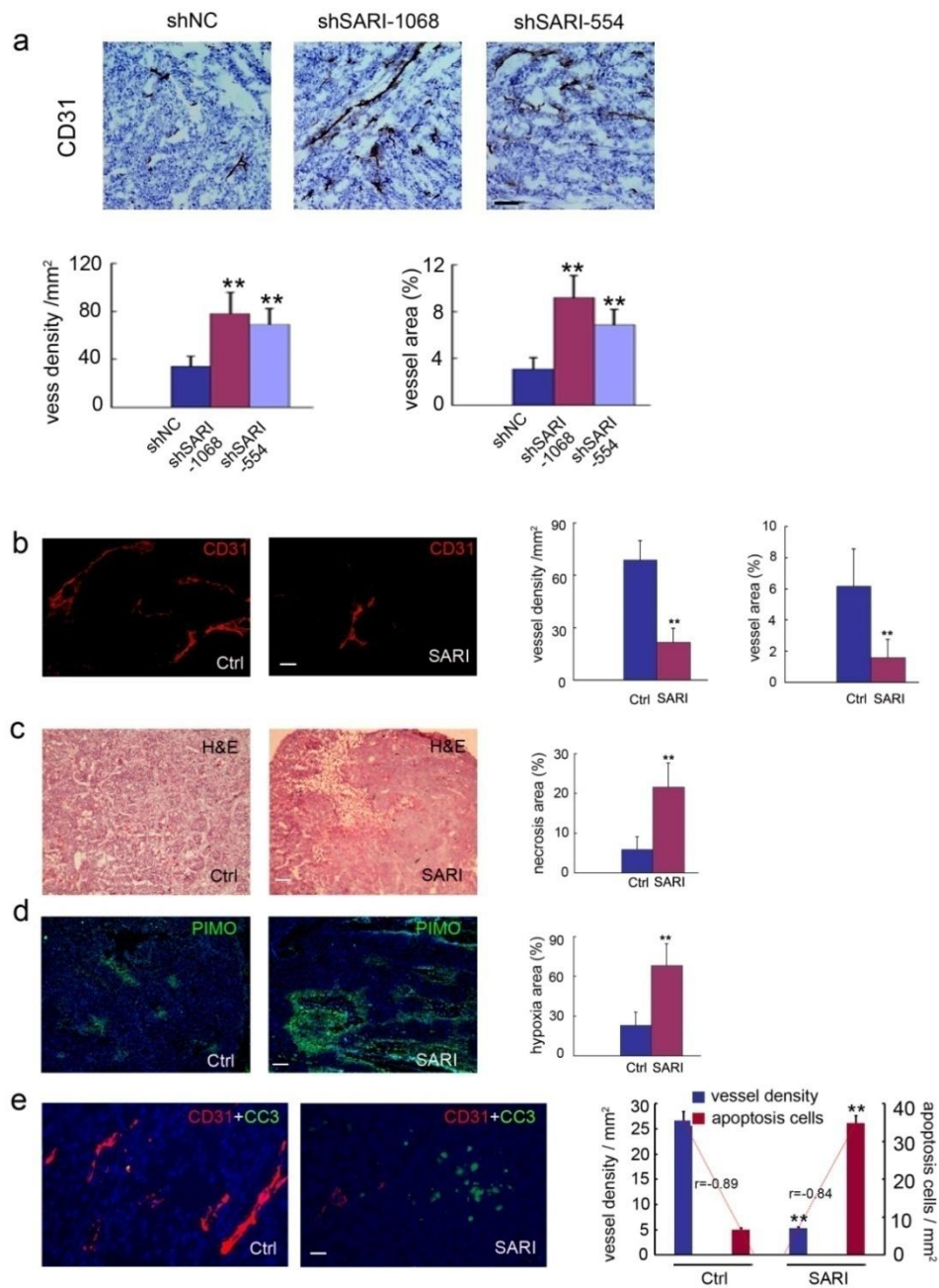


### Supplemental Figure 1 Expression of SARI in colon cancer cells.

(a-b) Reverse-transcription PCR (a) and Western blotting (b) was performed to evaluate SARI mRNA and protein level in colon cancer cell lines, and normal colon tissue was used as positive control, GAPDH was used as a loading control.

(c-d) Four shRNA target human SARI were constructed and inserted into lentivirus vector, scramble sequence was as the negative control (shNC) and SARI expression in HCT15 cells was determined by western blotting after lentivirus-shRNA infection.

Supplementary Figure-2 Deng



**Supplemental Figure 2 SARI inhibits angiogenesis and promotes necrosis in HCT116 tumors.**

(a) Staining for CD31 (red) in shNC, shSARI-1068 and shSARI-554-HCT15 tumors. Vessel density (/mm<sup>2</sup>; n=5; \*\*p< 0.01; ANOVA analysis) and vessel area (%; n=5; \*\*p< 0.01; ANOVA analysis) were quantified. Scale bar: 50  $\mu$ m.

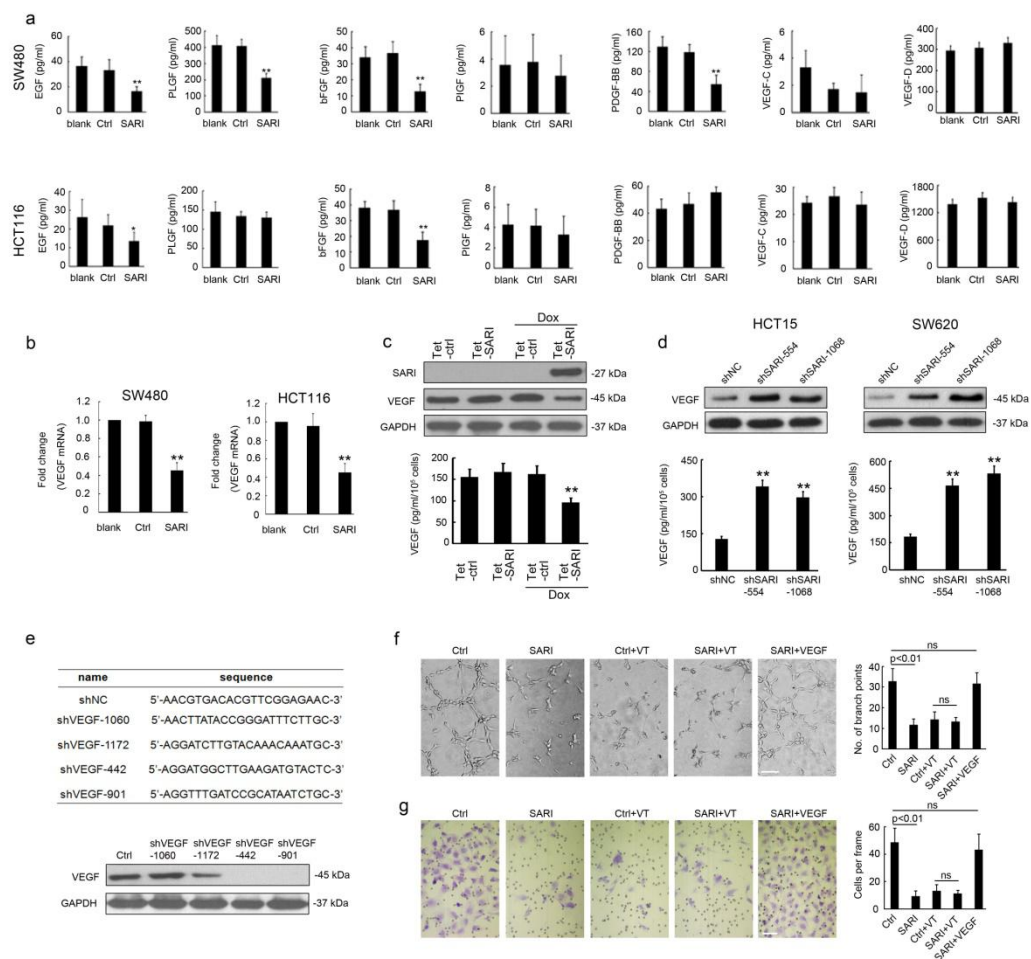
(b) Staining for CD31 (red) in ctrl and HCT116-SARI tumors. Vessel density ( /mm<sup>2</sup>; n=5; \*\*p< 0.01; Student's t test) and vessel area ( %; n=5; \*\*p< 0.01; Student's t test) were quantified. Scale bar: 50 μm.

(c) H&E staining showed more necrosis (c) in SARI than ctrlHCT116 tumors. Analysis of necrotic area (% of tumor area; Student's t test) at 25d (n=5; \*\*p< 0.01; Student's t test ) after tumor cell injection. Scale bar: 100 μm.

(d) Staining for pimonidazole (PIMO; green) in SARI and ctrl HCT116 tumors (d). Analysis of PIMO area (% of tumor area) (n=5; \*\*p< 0.01; Student's t test) after tumor cell injection. Scale bar: 100 μm.

(e) Staining for cleaved caspase-3 (green, CC3) and CD31 (red) in SARI and ctrlHCT116 tumors; apoptosis cells and vessel density was counted (n=5; \*\* p< 0.01; Student's t test). The correlation between CC3 and CD31 was analyzed, and the red line indicates the correlation (Pearson correlation analysis). DAPI staining for nucleus. Scale bar: 50 μm.

Supplementary Figure-3 Deng



### Supplemental Figure 3 VEGF mediates angiogenesis inhibition by SARI-controlled.

(a) EGF, PLGF, bFGF, PIGF, PDGF-BB, VEGF-C and VEGF-D exacted by SW480 and HCT116 cells were quantified by ELISA (n=3; \*p< 0.05; \*\*p<0.01; ANOVA analysis).

(b) VEGF mRNA expression using real time PCR in SW480 and HCT116 cells with or without stable expression of SARI(n=3; \*\*p<0.01; ANOVA analysis).

(c) Western blotting and ELISA were performed to determine the expression of SARI and VEGF after transfection with pLVX-Tet-SARI and pLVX-Tet-ctrl plasmid. Dox was added to induce plasmid expression. GAPDH as the loading control. (n=3; \*\*p< 0.01; ANOVA analysis).

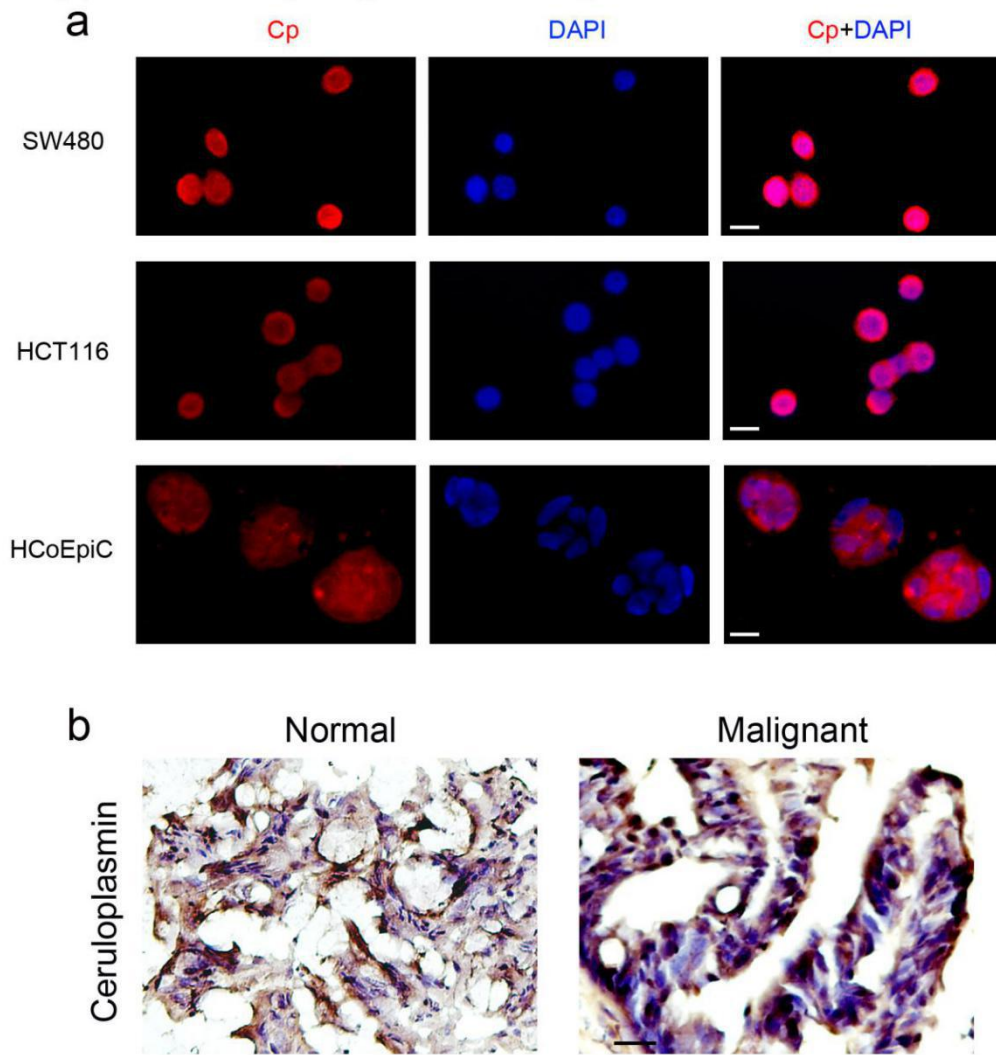
(d) Western blotting and ELISA were performed to determine the expression of VEGF after knockdown of SARI in HCT15 and SW620 cells. GAPDH as the loading control. (n=3; \*\*p< 0.01; ANOVA analysis).

(e) Four shRNA target human VEGF were constructed, scramble sequence was as the negative control (shNC) and VEGF expression in SW480 cells was determined by western blotting after shRNA transfection. GADPH as the loading control.

(f) Endothelial tube formation was estimated following the incubation of HUVECs with conditioned medium from SW480-ctrl, SW480-SARI, SW480-SARI+VEGF, SW480-ctrl+VEGF-trap and SW480-SARI+VEGF-Trip cancer cells. The number of branches was quantified (n = 5; p<0.01; ns, no significant difference; ANOVA analysis). Scale bar: 50  $\mu$ m.

(g) Representative photomicrographs of HUVECs that have invaded through Matrigel chambers after incubation with conditioned medium from SW480-ctrl, SW480-SARI, SW480-SARI+VEGF, SW480-ctrl+VEGF-trap and SW480-SARI+VEGF-Trip cancer cells for 72 hrs. Scale bar: 50  $\mu$ m. Quantification of HUVECs that have invaded through Matrigel chambers (n=5; p<0.01; ns, no significant difference; ANOVA analysis).

## Supplementary Figure 4-Deng

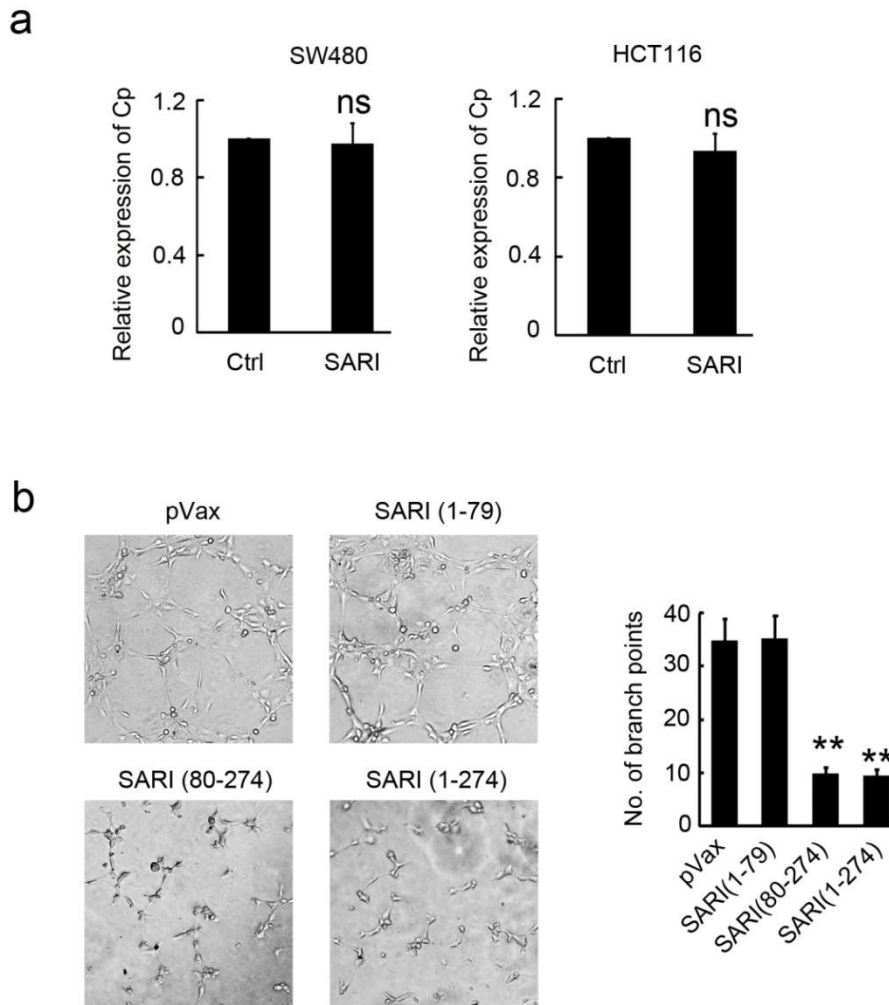


### Supplemental Figure 4 Cp distribution in normal and colon cancer cells and tissues

(a) Immunofluorescence was employed to stain Cp distribution in SW480, HCT116 and HCoEpiC cells. The DAPI indicated the nucleus. Scale bar: 20  $\mu$ m.

(b) IHC staining was performed to detect Cp distribution in malignant colonic tissues and adjacent normal colonic tissues from patients. Scale bar=50  $\mu$ m

## Supplementary Figure-5 Deng

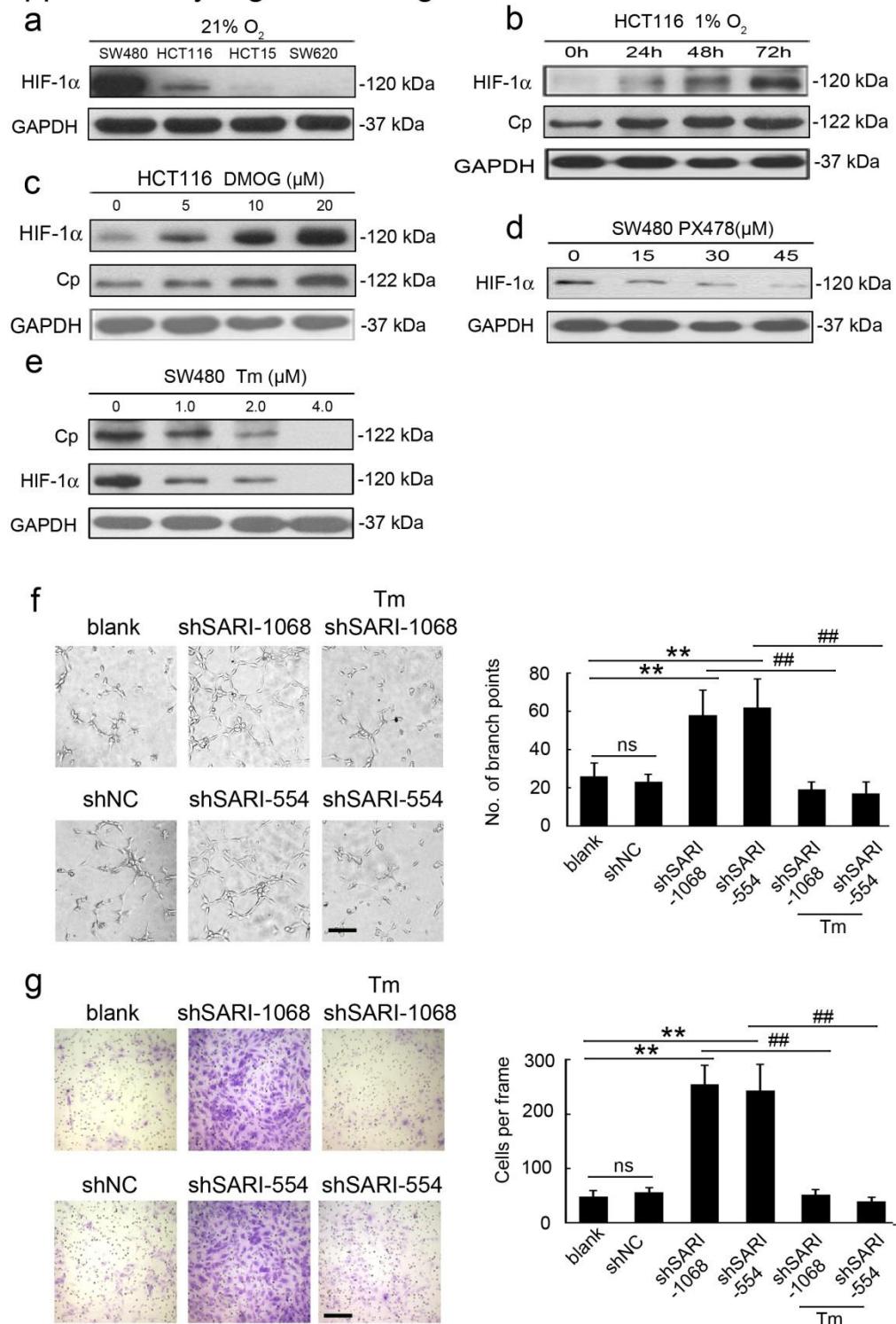


### Supplemental Figure 5 Stepwise deletion of SARI indicated that 80-274 amino acid of SARI takes the role of inhibiting angiogenesis

(a) Cp mRNA expression using real time PCR in SW480 and HCT116 cells with or without stable expression of SARI(n=3; \*\*p< 0.01 Student's t test).

(b) Endothelial tube formation was estimated following the incubation of HUVECs with conditioned medium from the SW480 cells after transfected with pVax, HA-SARI (encoding 1-79 aa), HA-SARI (encoding 80-274 aa) and HA-SARI (encoding 1-274 aa). The number of branches was quantified (n = 3; \*\*p<0.01; ANOVA analysis).

## Supplementary Figure 6-Deng



**Supplemental Figure 6 SARI inhibits HIF-1 $\alpha$  expression through directly targeting Cp.**

(a) Immunoblots of HIF-1 $\alpha$  expression in SW480, HCT116, HCT15 and SW620 cells. GAPDH as

the loading control.

(b) Immunoblots of HIF-1 $\alpha$  expression in HCT116 cells under hypoxia for 0, 24, 48 and 72 hrs, GAPDH as the loading control.

(c) Immunoblots of HIF-1 $\alpha$  expression in HCT116 cells after treatment with DMOG at the concentration of 0, 5, 10 and 20  $\mu$ M, GAPDH as the loading control.

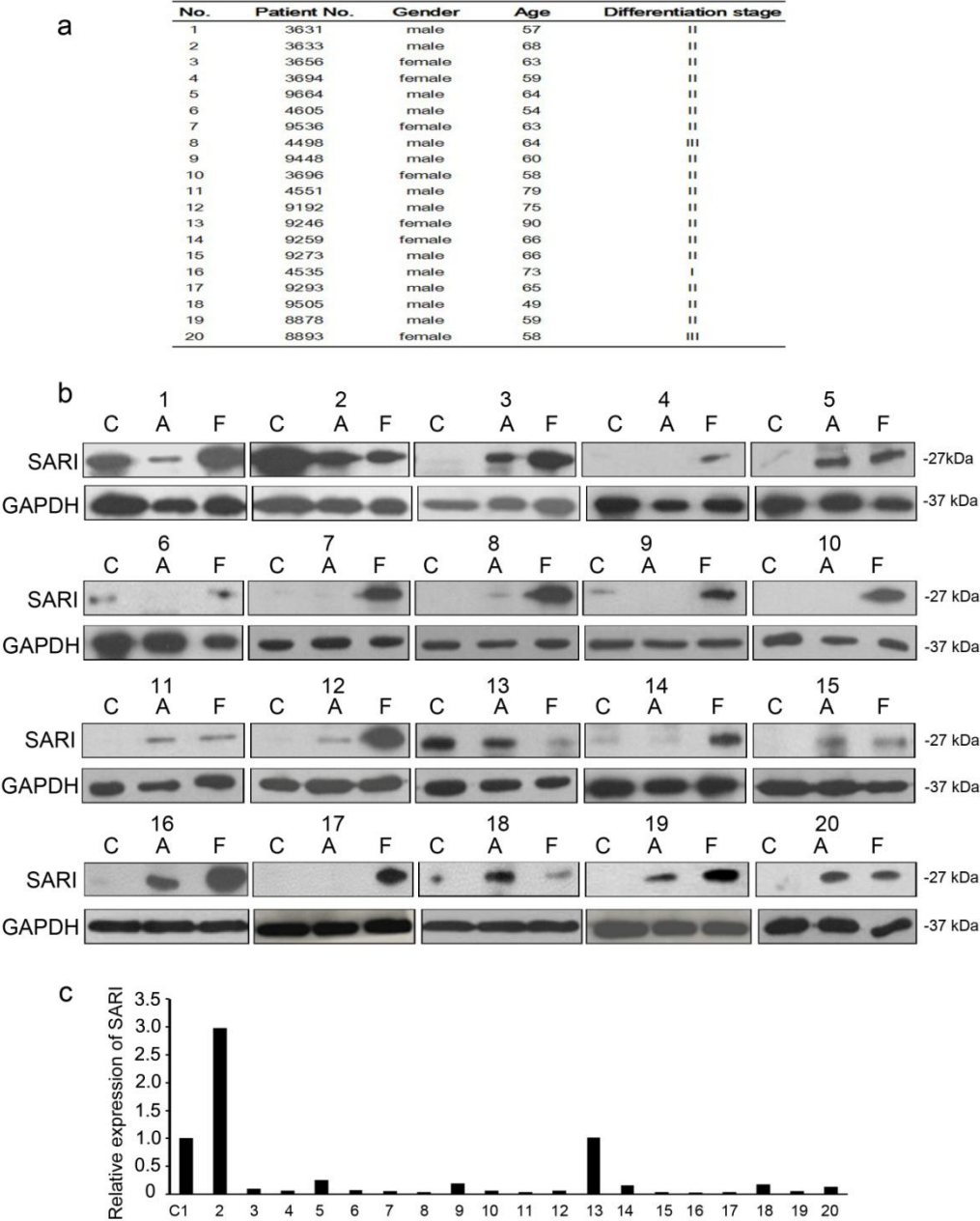
(d) Immunoblots of HIF-1 $\alpha$  expression in SW480 cells after treatment with PX478 at the concentration of 0, 15, 30 and 45  $\mu$ M, GAPDH as the loading control.

(e) Immunoblots of Cp and HIF-1 $\alpha$  expression in SW480 cells after treatment with the Cp inhibitor, tetrathiomolybdate (Tm), at the concentration of 0, 1.0, 2.0 and 4.0  $\mu$ M, GAPDH as the loading control.

(f) Endothelial tube formation was estimated following the incubation of HUVECs with conditioned medium from HCT15, HCT15-shNC, HCT15-shSARI-1068 and HCT15-shSARI-554 cells with or without Tm treatment (4.0  $\mu$ M). The number of branches was quantified (n=5; \*\*p< 0.01; ##p<0.01; ANOVA analysis). Scale bar: 50  $\mu$ m.

(g) Representative photomicrographs of HUVECs that have invaded through Matrigel chambers after incubation with conditioned medium from HCT15, HCT15-shNC, HCT15-shSARI-1068 and HCT15-shSARI-554 cells with or without Tm treatment (4.0  $\mu$ M). Quantification of HUVECs that have invaded through matrigel chambers (n = 5; \*\* p< 0.01; ##p<0.01; ANOVA analysis). Scale bar: 100  $\mu$ m.

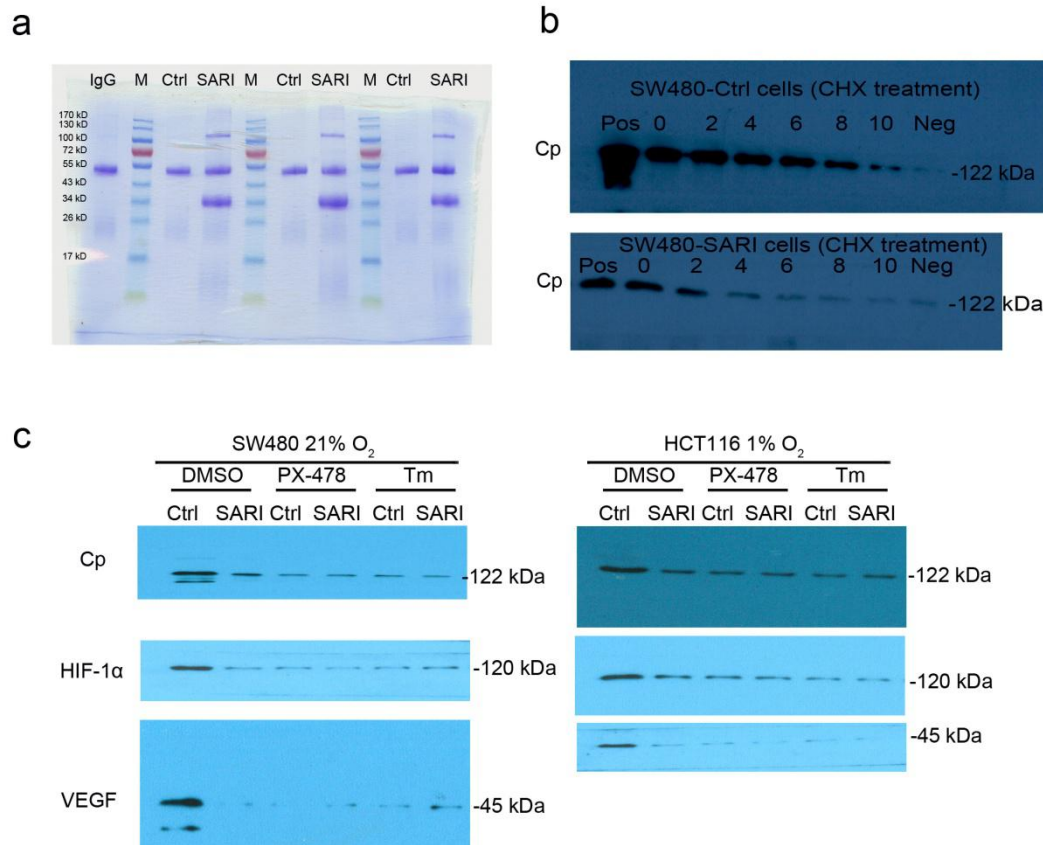
Supplementary Figure-7 Deng



**Supplemental Figure 7 SARI expression in 20 pairs of colon cancer tissues.**

- (a) The details of patients with colon cancer.
- (b) Immunoblots of SARI expression in colon cancer, adjacent and forward tissue of 20 patients with colon cancer, GAPDH as a loading control.
- (c) The density of SARI expression was measured by Image J software

## Supplementary Figure-8 Deng



### Supplementary Figure 8 Uncropped the most important western blot images.

(a) Uncropped scans of gels to Figure 4a. Co-immunoprecipitation products in SW480-Ctrl and SW480-SARI cells after adding anti-SARI antibody were separated by SDS-PAGE.

(b) Uncropped scans of western blots to Figure 4h. Measurement of Cp in SW480-Ctrl and SW480-SARI cell lysates harvested at 0, 2, 4, 6, 8, and 10 hours after the addition of CHX to arrest protein synthesis. (Pos, positive control; Neg, negative control)

(c) Uncropped scans of western blots to Figure 5b. Immunoblots of Cp, HIF-1 $\alpha$  and VEGF expression in SW480-ctrl and SW480-SARI cells with or without PX478 (45  $\mu$ M) or Tm (4.0  $\mu$ M) treatment under normoxia. Immunoblots of Cp, HIF-1 $\alpha$  and VEGF expression in HCT116-ctrl and HCT116-SARI cells with or without PX478 (45  $\mu$ M) or Tm (4.0  $\mu$ M) treatment under hypoxia.